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Progress and challenges in identifying molecular mechanisms underlying host and vector manipulation by plant viruses

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Plant virus infection fundamentally alters chemical and behavioral phenotypes of hosts and vectors. These alterations often enhance virus transmission, leading researchers to surmise that such effects are manipulations caused by virus adaptations and not just by-products of pathology. But identification of the virus components behind manipulation is missing from most studies performed to date. Here, we evaluate causative empirical evidence that virus components are the drivers of manipulated host and vector phenotypes. To do so, we link findings and methodologies on virus pathology with observational and functional genomics studies on virus manipulation. Our synthesis provides an overview of progress, areas of synergy, and new approaches that will lead to an improved mechanistic understanding of host and vector manipulation by plant viruses.

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Introduction

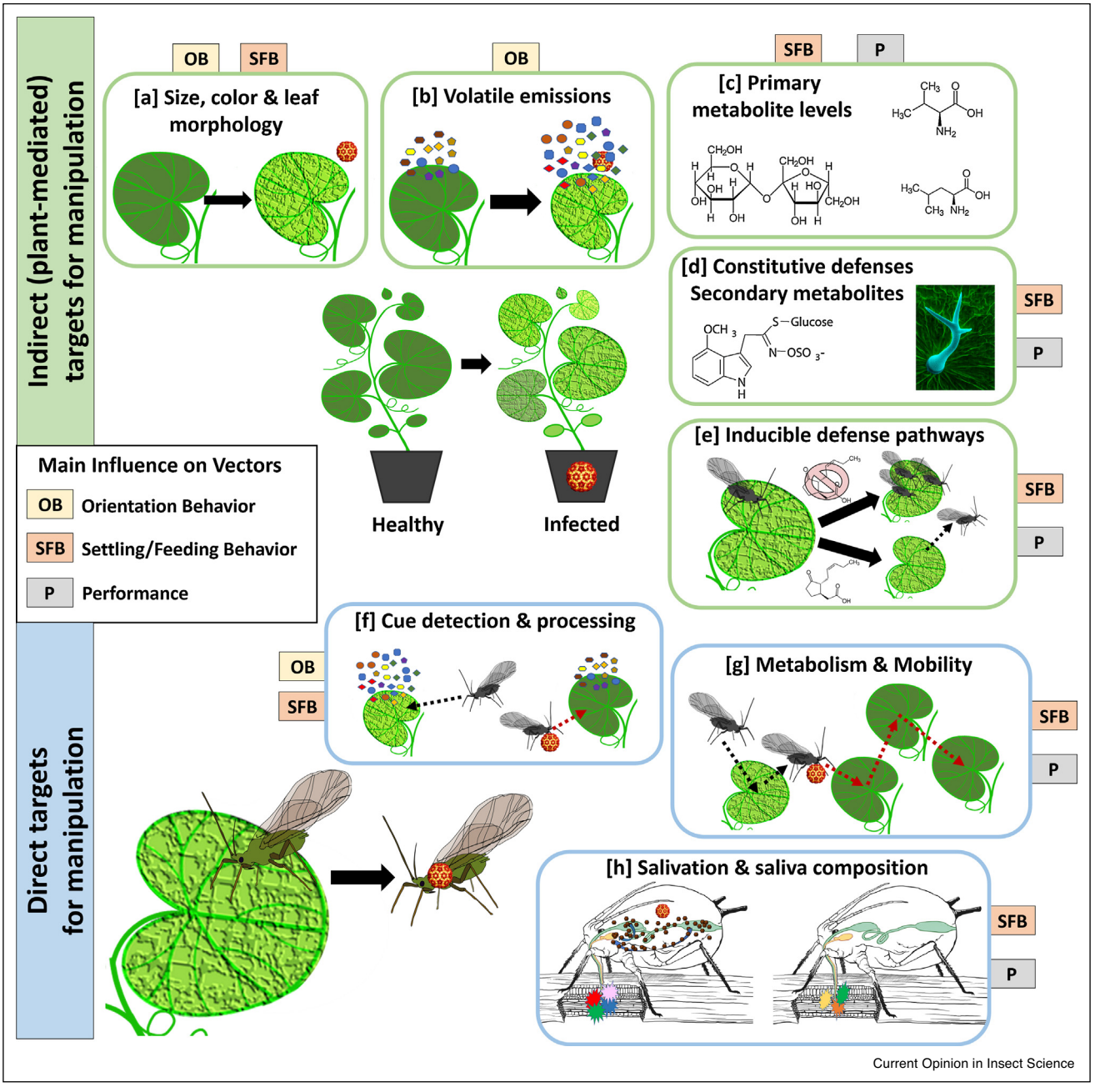
Manipulation of plant hosts and arthropod vectors has emerged as an important component of plant virus ecology and epidemiology. There are now numerous studies documenting changes in vector orientation behavior, settling and feeding behavior, and/or performance due to virus infection in host plants, most of which are expected to enhance virus transmission (recently reviewed in Ref. [1•]). This pathway for vector manipulation is *indirect* because the virus modifies insect behavior via changes in the physiology of the shared host plant resource (Figure 1). Most reports of putative plant virus manipulation fall into this category, but more recently, several studies have documented possible *direct* effects of viruses on vector behaviors relevant for transmission. Direct effects manifest as changes in vector behavior that occur following acquisition and retention of virions, and, like indirect effects, documented cases of direct effects tend to enhance the probability of virus transmission [2–4] (Figure 1). There are several excellent reviews summarizing putative instances of indirect and direct manipulation by plant viruses [1•,5–9], but we have only a nascent understanding of how viruses are controlling hosts and vectors. Here, we discuss possible pathways for host and vector manipulation based on knowledge of virus pathology from the virology literature, review progress toward pinpointing the virus components responsible for inducing manipulated phenotypes, and identify critical knowledge gaps and their implications for the broader fields of virus and vector ecology.

Defining manipulation in the context of constraints on plant virus evolution

To be categorized as 'parasite manipulation' a documented effect of a plant virus on its vector should satisfy, at minimum, two criteria [10–12]. First, it should result in enhanced virus transmission, or at least create conditions expected to enhance transmission given knowledge of how viruses are acquired and inoculated by vectors [1•]. Second, the effect(s) should be under genetic control of the virus, and, thus, subject to natural selection [10,11]. Accumulated evidence supporting the claim that plant viruses are manipulating insect vectors largely addresses the first criterion. For example, nearly all plant viruses examined thus far enhance the attractiveness of their host plants to vectors via changes in volatile odor compounds, visual appearance, or both of these phenotypic aspects (reviewed in Refs. [1•,7], see also Refs. [[1•],13–21] and Figure 1). This pattern is evident across diverse virus families and transmission mechanisms, which is expected given that increasing the probability of vector contacts with infected hosts is generally beneficial for pathogen spread [22,23,24•].

Reports of viruses manipulating plant palatability cues and vector feeding behavior are also well documented. Unlike virus-induced changes in long-range cues (volatiles, color), which uniformly favor enhanced vector attraction to infected hosts, virus effects on plant palatability tend to differ depending on the transmission mechanism of the virus under study. For example, viruses that are acquired through long periods of ingestion from

Figure 1

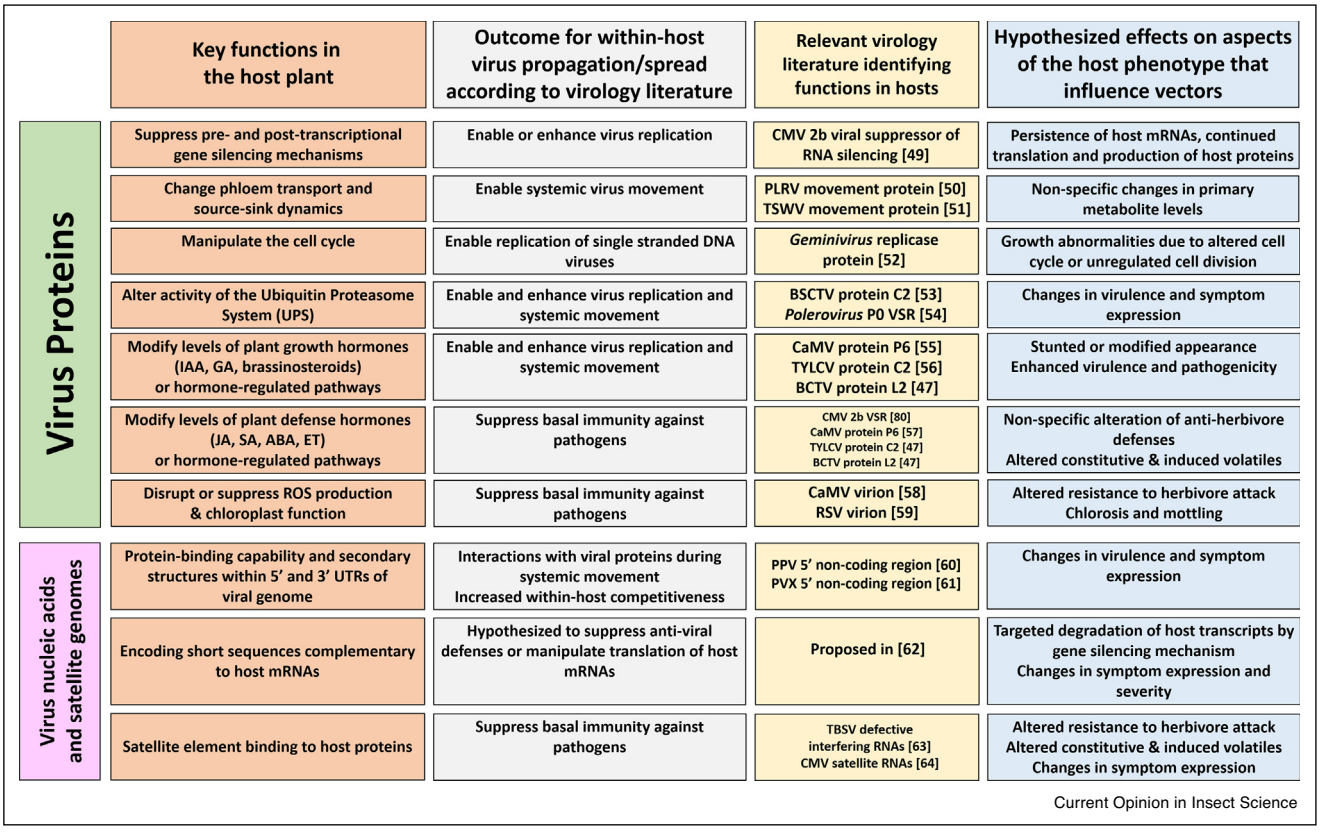


Aspects of the host phenotype (top — indirect effects in green boxes) and components of vector behavior and physiology (bottom — direct effects in blue boxes) that are frequently altered following virus infection or acquisition. Virus infection in plants typically modifies **(a)** the physical characteristics of plant parts; **(b)** production and release of volatile compounds [17]; **(c)** primary metabolites such as amino acids and sugars [38]; **(d)** constitutive defenses, including secondary metabolites (4-methoxy-indol-3-yl-methylglucosinolate and trichomes pictured here, trichome image by Heiti Paves© 2013) [38,42]; and **(e)** inducible defenses and phytohormones (salicylic acid and jasmonic acid pictured here) [89]. The lower portion of the figure presents possible mechanisms by which virus acquisition by vectors modifies preferences and physiology. These include virus effects on cue detection and processing, possibly through direct interactions of viruses with insect tissues **(f)** [2,3]; changes in metabolism and mobility following virus traversal of the midgut or virus replication in vector tissues **(g)** [114]; and virus effects on salivation behavior or the protein components of vector saliva **(h)** [102]. Circulative viruses reside in the salivary glands but their effects on vector saliva are not well studied. For each phenotypic aspect in both parts of the figure, tags indicate whether modifications are likely to alter vector orientation preferences, settling/feeding behavior, or performance. Orientation behavior (OB) refers to vector perception of, and responses to, long-range cues associated with identifying and contacting host plants (odor and visual aspects). Settling and feeding behavior (SFB) refers to the behavioral sequences necessary for assessing host palatability cues (nutrients, secondary metabolites, leaf toughness) and engaging in prolonged ingestion of plant sap or leaf tissue, all of which are components of virus acquisition and inoculation. Performance (P) refers to metrics of vector fecundity and survival over time during an extended interaction with a host plant and is important for virus transmission because vector numbers partially determine transmission rates [24•].

the host often increase host palatability and ease of accessing the tissues containing virions (e.g. phloem) (reviewed in Refs. [1•,7], see also Refs. [[1•],17,19,25–29] and Figure 1). This is expected to increase virus transmission because vectors will preferentially settle on infected plants, contact the tissue housing virions more rapidly, and, once reached, take up a larger number of virions from this tissue. Along with palatability, these same viruses also tend to increase host quality, which is expected to lead to enhanced production of vectors that will acquire and retain virions before dispersal (reviewed in Ref. [1•], see also Refs. [30–35] and Figure 1). In contrast, for viruses acquired and inoculated through brief probes of host tissue, there are many documented cases of reductions in host palatability and quality following virus infection (reviewed in Refs. [1•,36], see also Refs. [15,16,37–43]). This is consistent with expectations for manipulation by these pathogens because they are generally lost from the mouthparts if the vector does not engage in brief probing of tissues followed by dispersal [44–46]. By reducing host plant palatability and quality, rapidly acquired viruses could limit phloem sap ingestion (during which virions are lost) and encourage vector dispersal following virion acquisition.

Although transmission-mechanism specificity supports the idea that plant virus effects are adaptive and not uniform by-products of pathology, it does not address the second requirement to demonstrate that virus-induced changes in host phenotype, vector behavior, or vector performance are under genetic control of the parasite [11,12]. Plant viruses have some of the smallest

Figure 2



Mechanisms of symptom induction by viruses and hypothesized links to manipulation of plant hosts and vector behavior. This figure draws parallels between studies from the virology literature that establish a genetic basis for symptom induction and possible pathways by which the virus components responsible for symptoms could *also* alter aspects of the host phenotype that influence vector behavior. Along the left edge, we highlight two types of virus components (proteins and nucleic acids) that have activity in the host plant. The first column (orange) describes functions of these components (how they interact with the host) and the second column (white) describes the outcome for the virus in terms of replication and systemic spread within a single host plant. The third column (yellow) provides examples of virus components that induce symptoms in host plants via the mechanisms listed in columns one and two. The fourth column extends these examples from the virology literature to develop hypotheses about how the virus components listed could be co-opted to manipulate the host phenotype in ways that enhance transmission by vectors. Viruses listed in column three are *Cucumber mosaic virus* (CMV, *Bromoviridae*), *Potato leafroll virus* (PLRV, *Luteoviridae*), *Tomato spotted wilt virus* (TSWV, *Tospoviridae*), *Beet severe curly top virus* (BSCTV, *Geminiviridae*), *Cauliflower mosaic virus* (CaMV, *Caulimoviridae*), *Tomato yellow leaf curl virus* (TYLCV, *Geminiviridae*), *Beet curly top virus* (BCTV, *Geminiviridae*), *Rice stripe virus* (RSV, *Tenuivirus*), *Plum pox virus* (PPV, *Potyviridae*), *Potato virus X* (PVX, *Alfaflexiviridae*), *Tomato bushy stunt virus* (TBSV, *Tombusviridae*).

genomes of any organism (roughly 4–20 kb). Nonetheless, viruses with just a few genes are capable of inducing drastic changes in the physiology of their host plants [47–58,59•,60–64] (Figure 2). Viruses also have genetically encoded adaptations for interacting with their vectors following acquisition, including proteins that facilitate binding to the cuticle (stylet, foregut), crossing cellular membranes, trafficking within hemolymph, and invading and replicating in vector tissues [65–70,71•,72,73,74,75•,76,77,78•] (Figure 3). It is possible that virus traits facilitating these intimate associations with vectors could be co-opted to induce behavioral changes that enhance transmission (Figures 1 and 3). Using functional genomics, the roles of various virus proteins and genetic elements have been measured by quantifying the impacts of viral mutations on virus-host and virus-vector interactions (Figures 2 and 3). Protein-coding genes and other genetic elements that enhance within-host colonization and spread, or retention and colonization of the vector, are presumed to be under strong selection to maintain, and possibly improve, these functions. It is within this restrictive fitness landscape that virus traits enabling manipulation of hosts and vectors must evolve. Inevitably, this will involve one or more components that are *already* performing an essential role for host or vector exploitation (see e.g. in Figures 2 and 3). Thus, genetic changes in viruses that enable manipulation of host phenotypes and vector behavior should do so without negatively impacting other protein functions essential for virus replication and spread within a host or virus acquisition and retention in vectors.

Genetic basis of indirect (plant-mediated) effects

Alterations of host phenotypes in response to virus infection can be considered as a form of symptom expression, an aspect that is well studied because symptoms are strongly linked to virus impacts on plant fitness and fruit

Figure 3

Virus component	Functions in vectors according to virology literature	Relevant virology literature identifying functions in vectors	Hypothesized pathways for virus components to directly alter vector behavior
Virus-encoded glycoproteins Virions and/or capsid proteins (circulative, propagative viruses)	Glycoproteins interact with midgut epithelial cell receptors to facilitate virion endocytosis. Act as a "helper component" Essential for completion of persistent propagative life-style in the vector	TSWV Gn glycoprotein [66-68] MMV G glycoprotein [69] RSV NSvc2 glycoprotein [70] TSWV [71]	Initiation of intracellular cascades through interactions with G protein receptors (glycoproteins) Activation of immune responses & alteration of transport functions
Proteins involved in virus replication within vector tissues (circulative, propagative viruses)	Generation and maturation of granular and tubular structures that contain virus particles in insect cells Facilitate replication of the genome and packaging of virions Form cell-to-cell movement-related tubules Induce membrane fusion in insect host cells	RDV P2 capsid protein [72] RDV Pns10 protein [70] SRBSDV P5, P6, P9-1 proteins [73] RSV NS4 protein [74] TSWV [71]	Invasion, replication, and movement within ganglia and nervous tissue associated with salivary glands Changes to cell function and gene expression caused by granules/tubules Modification of insect defense pathways, signaling pathways, lipid metabolism, stress responses in all cell types used for virus replication or transport.
Virions and coat proteins (circulative, non-propagative viruses)	Protect and transport viral nucleic acids Enable virion binding to insect midgut cells (e.g. via ephrin receptors) Enhance virion uptake by vector gut epithelial cells Enable virion transport to, and entry within, salivary glands	TYLCV [75] Luteoviridae capsid proteins (ephrin receptors) [76 & 78] PEMV [77]	Perturbations to cellular transport mechanisms targeting macromolecules Alteration of gut epithelial cell cycle Activation of humoral and cellular immune responses Changes in production of effector proteins in the salivary glands

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Plant virus components that enable or enhance invasion and replication within arthropod vectors, and possible pathways by which components could be co-opted for manipulating vector behavior. This figure summarizes what is known about how circulative viruses (those that invade vector tissues) alter the physiology of vectors following acquisition (columns one and two) and provides examples from the virology literature. We used this summary to develop hypotheses about how the identified virus components could be co-opted for direct manipulation of vector behavior (column four). In the first column, the term 'propagative' indicates viruses that also replicate in vector tissues, while 'non-propagative' indicates viruses that invade vector tissues (e.g. salivary glands), but do not replicate. Viruses included in column three are: *Tomato spotted wilt virus* (TSWV, *Tospoviridae*), *Maize mosaic virus* (MMV, *Rhabdoviridae*), *Rice stripe virus* (RSV, *Tenuivirus*), *Rice dwarf virus* (RDV, *Reoviridae*), *Southern riceblack-streaked dwarf virus* (SRBSDV, *Reoviridae*), *Tomato yellow leaf curl virus* (TYLCV, *Geminiviridae*), *Pea enation mosaic virus* (PEMV, *Luteoviridae*).

quality. From virology studies, it is evident that there are many pathways by which plant virus proteins could both promote infection in a host plant and modify the expression of symptoms in ways that alter interactions with vectors (Figure 2). This body of work also provides evidence that symptom expression is often *not* linked to the main function that a virus protein performs during invasion and exploitation of the host plant (Figure 2). For instance, targeted mutation of *Cucumber mosaic virus* (CMV, *Bromoviridae*) 2b protein revealed that it can control the expression of host symptoms (mottling, leaf deformations) independently of its functions in facilitating virus accumulation and systemic movement through suppression of the host's RNA silencing mechanism, which doubles as a natural antiviral defense [79,80]. This example, and others described in Figure 2, support the hypothesis that plant viruses can evolve to manipulate host phenotypes and vector behavior *without* compromising within-host replication and spread. A range of phenotypic changes in hosts have been associated with putative instances of virus manipulation of vector behavior and performance (Figure 1) and these overlap significantly with phenotypic changes (symptoms) induced by specific virus proteins (Figure 2).

Despite there being many predicted routes for indirect manipulation of vectors (Figures 1 and 2), so far, conserved plant defense pathways appear to be the primary targets of virus components implicated as drivers of manipulated phenotypes (Table 1). These components include a viral protease [81,82,83••], viral suppressors of RNA silencing [42,84••,85–87], a viral replicase [42], an RNA-dependent RNA polymerase [42], a nuclear shuttle protein [88], and a satellite DNA element external to the main virus genome [88–91]. Several of these components act on the jasmonic acid (JA) pathway, activation of which in uninfected plants normally results in the production of defenses against herbivores and some pathogens [92]. Among begomoviruses purported to manipulate host phenotypes and whitefly vectors, the *Tomato yellow leaf curl china virus* (TYLCCV) βC1 satellite (a DNA element that is encapsidated with the virus genome, but is external to it) and the *Cabbage leaf curl virus* (CaLCuV) BV1 nuclear shuttle protein both function to suppress JA-regulated defenses by binding MYC2 transcription factors [88–91]. In this case, the core functions of these virus components as promoters of within-host replication (by weakening antiviral defenses) and the ancillary functions in manipulation of vector behavior are collinear. And even though the βC1 gene is external to the virus genome, the TYLCCV βC1 protein localizes and behaves similarly to the genome-encoded CaLCuV BV1 protein [88]. Suppression of JA-regulated defenses by both βC1 and BV1 enables pathogen replication and spread within a host [47] and this *same* function also results in the suppression of defenses against whitefly vectors, which enhances their attraction to, and settling and feeding on, infected hosts (Table 1). Since begomoviruses are only acquired during long-term phloem ingestion, settling and feeding in the phloem for several hours are necessary for vectors to become viruliferous.

Cucumber mosaic virus (CMV) also increases host attractiveness to vectors, but unlike begomoviruses, CMV tends to *decrease* palatability via effects on within-plant cues, which is expected to increase transmission efficiency of this non-persistently transmitted virus by encouraging aphid vectors to acquire virions during superficial probing, and disperse before virions are lost from their mouthparts [15,16]. Like begomoviruses, CMV also encodes a protein (2b) that interacts directly with the JA pathway to augment host immunity and alter host-vector interactions, but in this case, the manipulative function appears to be independent of 2b activity as a viral suppressor of the host's RNA silencing mechanism, an antiviral defense (Table 1) [84••]. This was demonstrated by Wu *et al.* [84••] in a recent study implicating the 2b protein as the virus component responsible for rendering CMV-infected plants more attractive to aphid vectors via suppression of JA-regulated defenses. 2b has also been implicated in production of an unpalatable phenotype in CMV-infected plants, which aphid vectors encounter upon landing and probing tissues, and which contributes to the efficient transmission of the CMV pathogen [42]. In this case, 2b augments production of an antibiotic compound toxic to aphids, and two other virus proteins, the 1a replicase and 2a RNA-dependent RNA polymerase, act in concert to limit the toxic effects of 2b, resulting in an overall antixenotic phenotype that encourages dispersal of viruliferous vectors [42] (Table 1). It is interesting to note that the overall effects of CMV on host phenotypes, and the individual effects of different variants of the 2b protein, are not ubiquitous and appear to be host-dependent [16,42,87] (Table 1).

Host- and vector-specific effects of virus components are also evident in other pathosystems. The Potyviridae are important agricultural pathogens, and two species, *Potato virus Y* (PVY) and *Turnip mosaic* virus (TuMV) are well-studied from a functional genomics perspective across multiple hosts. For example, in a series of elegant studies on TuMV infecting *Nicotiana benthamiana* and *Arabidopsis thaliana*, it was determined that this virus enhances performance of its aphid vector on infected host plants via effects of the Nuclear-Inclusion a-Protease (NIa-Pro) on ethylene production and ethylene-regulated defenses against aphids (callose tissue deposition) (Table 1). For TuMV, this enhancement has been linked to increased opportunities for virus transmission [81]. A fascinating finding is that NIa-Pro must relocalize from the cytoplasm and nucleus to the vacuole in order to induce the observed effects, and it only does so when a competent aphid vector feeds on the host [83••]. NIa-Pro from PVY behaves in the same way in *N. benthamiana*, suggesting a

Table 1

Virus components implicated as drivers of vector behavioral manipulation via indirect effects (green) and direct effects (blue)

Pathosystem (virus, host, vector)	Virus effects	Virus component implicated	Core function	Novel function to enable vector manipulation	References
TYLCCV plus beta-satellite *Nicotiana tabacum, N. benthamiana, Arabidopsis thaliana* *Bemisia tabaci*	Vector orientation preference for infected hosts, settling preference for infected hosts (PC virus)	βC1 (satellite DNA outside of the main genome)	Suppresses JA pathway to enhance virus replication and host exploitation	Binds MYC2 transcription factor to compromise activation of MYC2-regulated terpene synthases, suppress JA-mediated defenses against vectors	[88–91]
CaLCuV *A. thaliana B. tabaci*	Predicted to increase vector performance and settling on infected plants (PC virus)	DNA-B BV1	Nuclear shuttle protein, interacts with movement protein for cell-to-cell movement	Binds MYC2 transcription factor to compromise activation of MYC2-regulated terpene synthases	[88]
TuMV, *N. benthamiana* *Myzus persicae*	Greater vector fecundity on infected versus healthy hosts, settling preference for infected hosts (NP virus)	NIa-pro	Cleaves the viral polyprotein at seven of the nine cleavage points.	Stimulates ET production, expression of ET-regulated genes that suppress deposition of callose tissue, relocalization to the vacuole in presence of aphids	[81,82,83••]
PVY *N. benthamiana* *M. persicae*	Greater vector fecundity on infected versus healthy hosts (NP virus)	NIa-pro	Cleaves the viral polyprotein at seven of the nine cleavage points.	Relocalizes to the vacuole, functions presumed similar to TuYV NIa-Pro	[83••]
PVY *N. benthamiana* *M. persicae*	Reduced vector fecundity on infected versus healthy hosts (NP virus)	HC-Pro	Sequesters small RNAs in the plant, binds HEN1 host protein; in vector acts as a helper component to attach virions to mouthparts	Transgenic expression increases vector fecundity, but this effect is not evident in natural PVY infections in *N. benthamiana*.	[85]
CMV *A. thaliana* *M. persicae*	Vector orientation preference for infected hosts, preferential dispersal from infected hosts, reduced performance (NP virus)	1a, 2a, and 2b	1a is the viral replicase, 2a is the RNA-dependent RNA polymerase, and 2b is the viral suppressor of RNA silencing (binds small RNAs, inhibits AGO1 protein)	2a protein elicits host defenses and antixenosis, 2b protein elicits host defenses and antibiosis, 1a protein moderates antibiotic activity of 2b to ensure aphid survival	[42]
CMV *N. tabacum,* *M. persicae*	Increased phloem ingestion by vectors, increased vector fecundity on infected versus healthy plants (NP virus)	2b	Viral suppressor of RNA silencing (binds small RNAs, inhibits AGO1 protein)	Suppresses JA-mediated defenses, enhances SA accumulation in response to CMV infection	[86]
CMV *A. thaliana,* *M. persicae*	Vector orientation preference for infected versus healthy hosts (NP virus)	2b	Viral suppressor of RNA silencing (binds small RNAs, inhibits AGO1 protein)	Inhibits JA signaling by repressing degradation of JA-regulatory proteins. This inhibition leads to increased host attractiveness to vectors	[84••]
BYDV *Triticum aestivum* *Rhopalosiphum padi*	Non-viruliferous aphids prefer infected plants, viruliferous aphids prefer healthy plants (PC virus)	Virion	Encapsidation of viral RNA during within-host and between-host spread, enable virion movement from gut to salivary tissues in vector insects	Retention of the virion results in reversal of aphid preference for infected hosts. Implication of virion alone is made possible by *in-vitro* virus acquisition assays.	[3]
TSWV *Datura stramonium* *Frankliniella occidentalis*	Viruliferous thrips feed more than non-viruliferous thrips (PCP virus)	Unknown	Involves virus-induced changes occurring during invasion and replication in midgut, visceral muscle, and salivary glands in juvenile stage	Virus acquisition as a first instar, and subsequent propagation throughout vector development, increases adult feeding frequency and intensity	[102]

Table 1 (*Continued*)

Pathosystem (virus, host, vector)	Virus effects	Virus component implicated	Core function	Novel function to enable vector manipulation	References
TYLCV *Solanum lycopersicum* *B. tabaci*	Viruliferous whiteflies salivate more in the phloem, feed more often. Viruliferous males have faster development. (PC virus)	Virion	Encapsidation of viral RNA during within-host and between-host spread, enable virion movement from gut to salivary tissues in vector insects	Retention of virions is associated with behavioral and life-history shifts. Controls for acquisition access period include healthy hosts of the same age. Insects reared on non-host for virus.	[99,100]
ToSRV *Solanum lycopersicum* *B. tabaci*	Viruliferous whiteflies prefer volatiles emitted from mock-inoculated plants (PC virus)	Virion	Encapsidation of viral RNA during within-host and between-host spread, enable virion movement from gut to salivary tissues in vector insects	Retention of virions is associated with behavioral shifts. Controls for acquisition access period include healthy hosts of the same age. Insects reared on non-host for virus.	[20]

Abbreviations: TYLCCV (*Tomato yellow leaf curl china virus*, *Geminiviridae*), CaLCuV (*Cabbage leaf curl virus*, *Geminiviridae*), TuMV (*Turnip mosaic virus*, *Potyviridae*), PVY (*Potato virus Y*, *Potyviridae*), CMV (*Cucumber mosaic virus*, *Bromoviridae*), BYDV (*Barley yellow dwarf virus*, *Luteoviridae*), TSWV (*Tomato spotted wilt virus*, *Tospoviridae*), TYLCV (*Tomato yellow leaf curl virus*, *Geminiviridae*), ToSRV (*Tomato severe rugose virus*, *Geminiviridae*). PC virus = persistently transmitted circulative virus (circulates but does not replicate in vector), PCP virus = persistently transmitted circulative propagative virus (circulates and replicates in vector), NP virus = non-persistently transmitted virus (no circulation or replication in vector, temporary retention on stylet).

conserved function for NIa-Pro in modifying interactions between plant hosts and competent aphid vectors [83••]. However, TuMV, PVY, or their respective NIa-Pro proteins, all failed to produce the same effects in *Nicotiana tabacum* [83••], and a different isolate of PVY than that used by Bak *et al.* [83••] was also shown to reduce fecundity of aphids when infecting *N. benthamiana* [85]. This variation is intriguing because it prompts us to consider the evolution of manipulative virus traits, and the molecular pathways by which they induce phenotypic changes, in an ecological context that includes scenarios where such traits might be maladaptive.

Genetic basis of direct effects

Direct manipulation of host behavior to enhance transmission is well documented for trophically-transmitted eukaryotic parasites [12,93]. Although they are far less complex lifeforms, circulative plant viruses have similarly intimate associations with their insect vectors [94–96,97•]. For example, circulative, non-propagative viruses cross multiple membrane barriers on the path to the salivary glands, and circulative propagative viruses additionally replicate within various vector tissues. As with virus-plant interactions (Figure 2), the virus components that enable transport and propagation within vectors also have broad effects on vector cell morphology, signaling pathways, neurology, immunity, and other physiological aspects (see examples in Figure 3). Functional genomics approaches and protein-protein interaction studies have revealed the specific virus components mediating these changes in multiple pathosystems, while transcriptomic and proteomic analyses of different vector tissues following virus acquisition elucidate interaction networks perturbed by virus invasion or replication (see examples in Figure 3). At present, studies documenting direct effects of plant viruses on vector behavior have not employed functional genomics approaches, but instead focused on characterizing effects of wild-type viruses.

We identified eight reports of transmission-conducive changes in vector behavior following acquisition of a circulative, non-propagative virus (Luteoviridae [[1•],3,19], Geminiviridae [20,98•,99–101], and six reports of changes in vector behavior following acquisition and/or replication of a circulative, propagative virus (Tospoviridae [102,103], Reoviridae [104–106], and a Tenuivirus [107]). Across these reports, virus acquisition by the vector is associated with changes in the degree of preference for orienting toward, or settling and feeding on, infected versus healthy hosts. In general, viruliferous vectors tend to prefer healthy hosts, and non-viruliferous vectors tend to prefer infected hosts. This is predicted to enhance virus spread [4,24•], leading researchers to propose that observed shifts in vector preference are the product of adaptations on the part of the virus. However, most of the studies cited above used vectors that were made viruliferous through rearing for *multiple generations*

on host plants infected with the virus under study. Given that virus-infected plants undergo significant changes in physiology, nutrition and defense status [1•] (Figure 1), for over half of the 'direct effects' reports published thus far, differences in vector behavior cannot be attributed solely to the presence or absence of virions within vectors.

A subset of studies incorporated methods to separate host-mediated from direct virus effects (Table 1). Ingwell *et al.* [3] verified that acquisition of purified BYDV virions from artificial diet was sufficient to induce a shift in vector settling preferences from infected plants to healthy plants. However, even this approach does not fully exclude a role for host proteins, as a previous study with a related virus found that phloem proteins remain attached to the virion surface during purification, and their presence increased the virus transmission rate [108]. Moreno-Delafuente *et al.* [99], Maluta *et al.* [100] and Fereres *et al.* [20] took a different approach by deriving whitefly vectors from the same colony and allowing them a 72 hour feeding period on same-age tomato plants with and without infection by different begomoviruses (ToSRV or TYLCV). Whiteflies were then used directly in electrical penetration graphing (EPG) experiments to measure probing and feeding behavior, preference assays, or transferred to non-host plants of the viruses to track insect survival and development. By standardizing acquisition times and methods, these studies provide evidence that intact virions are responsible for inducing changes in vector behavior following acquisition (Table 1), but it is not clear *when* following acquisition these changes take place (i.e. following invasion of midgut cells, movement to the hemocoel, or invasion of salivary glands). Among circulative propagative viruses, only one study clearly eliminated host carryover effects. Stafford *et al.* [102] allowed thrips vectors to acquire the circulative, propagative TSWV pathogen as first instars during a 24 hour feeding period, and subsequently reared vectors on green bean pods (a non-host for the virus). Adults were used for EPG recordings, which showed that TSWV infection in male thrips increased feeding behaviors conducive to virus inoculation. By temporally separating acquisition from virus effects, this study demonstrates that sex-specific alterations in behavior during the adult stage are linked with virus propagation within the vector during the juvenile stage. However, to date, no study describing direct effects has identified a functional explanation for the behavioral changes observed in vectors.

Outlook

There are now over 100 peer-reviewed publications reporting putative instances of vector manipulation by a plant virus, and the number of publications on this topic is growing on a monthly basis. Identification of the virus components underlying manipulations, and the pathways by which they perturb host and/or vector physiology, are the critical elements missing from most of these studies. Without causative empirical evidence that virus-encoded genes are the drivers of manipulation, many reports remain largely descriptive, with no way to rule out by-products of pathology as sources of supposedly manipulated phenotypes. Although establishing the mechanistic basis of virus manipulations is challenging, greater availability of host and vector genomic resources, and affordable technologies, are enabling approaches that before seemed unfeasible. For example, to elucidate virus pathology, virologists are now incorporating fine-scaled timelines in morphological, proteomic and transcriptomic studies [95,97•,109–111]. And there have also been major advancements in techniques for localizing viruses and their proteins in hosts and vectors [112]. By monitoring changes in tissue tropism and gene interaction networks over time, along with virus invasion and propagation, virologists can track which genes are turned on and off during key transitions in host or vector life stages and disease progression to gain insight into the molecular basis of virus pathology.

Among studies on host and vector manipulation, temporal aspects have been largely ignored, with most mechanistic studies targeting one time point for describing molecular differences between infected and uninfected hosts. But incorporating temporal profiles and molecular monitoring into studies on virus manipulation will help generate hypotheses regarding virus components involved (Figures 2 and 3) and give researchers new tools for confirming the presence of manipulative effects that influence vector behavior or life history traits. Hypotheses can be further tested using functional genomics approaches already employed for studying virus pathology (targeted mutagenesis, transient or transgenic expression of virus proteins) [83••,84••] and macromolecule or protein interaction studies [48,113]. Ideally, this work should also explore possible pleiotropic effects of alterations in the virus components implicated as drivers of manipulated phenotypes. Since most plant viruses infect multiple hosts, this could be accomplished by quantifying phenotypic shifts induced by virus components across several hosts or vectors to derive hypotheses about pathways for the evolution of manipulative traits in ecologically complex environments. Considering the number of studies already published (recently reviewed in Ref. [1•]) and the implications of virus manipulation for disease spread [4,24•], mechanistic studies must be a priority moving forward. Achieving an understanding of the genetic basis of plant virus manipulation will thus require extensive collaboration among researchers in the fields of virology, plant biology, ecology, and entomology.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. • Mauck KE, Chesnais Q, Shapiro LR: **Evolutionary determinants of host and vector manipulation by plant viruses**. *Adv Virus Res* 2018, **101**:189-250.

This is an in-depth synthesis of the current evidence of vector manipulation by plant viruses in the context of environmental and molecular factors constraining virus evolution. The authors also identify current gaps in the literature and new technologies that could be employed to fill these gaps.

2. Rajabaskar D, Bosque-Pérez NA, Eigenbrode SD: **Preference by a virus vector for infected plants is reversed after virus acquisition**. *Virus Res* 2014, **186**:32-37.

3. Ingwell LL, Eigenbrode SD, Bosque-Pérez NA: **Plant viruses alter insect behavior to enhance their spread**. *Sci Rep* 2012, **2**:1-6.

4. Roosien BK, Gomulkiewicz R, Ingwell LL, Bosque-Pérez NA, Rajabaskar D, Eigenbrode SD: **Conditional vector preference aids the spread of plant pathogens: results from a model**. *Environ Entomol* 2013, **42**:1299-1308.

5. Bosque-Pérez NA, Eigenbrode SD: **The influence of virus-induced changes in plants on aphid vectors: insights from luteovirus pathosystems**. *Virus Res* 2011, **159**:201-205.

6. Blanc S, Michalakis Y: **Manipulation of hosts and vectors by plant viruses and impact of the environment**. *Curr Opin Insect Sci* 2016, **16**:36-43.

7. Mauck KE, De Moraes CM, Mescher MC: **Effects of pathogens on sensory-mediated interactions between plants and insect vectors**. *Curr Opin Plant Biol* 2016, **32**:53-61.

8. Casteel CL, Falk BW: **Plant virus-vector interactions: more than just for virus transmission**. *Current Research Topics in Plant Virology*. Cham: Springer; 2016, 217-240.

9. Eigenbrode SD, Bosque-Pérez N, Davis TS: **Insect-borne plant pathogens and their vectors: ecology, evolution, and complex interactions**. *Annu Rev Entomol* 2018, **63**:169-191 http://dx.doi.org/10.1146/annurev-ento-020117-043119.

10. Poulin R, Maure F: **Host manipulation by parasites: a look back before moving forward**. *Trends Parasitol* 2015, **31**:563-570.

11. Heil M: **Host manipulation by parasites: cases, patterns, and remaining doubts**. *Front Ecol Evol* 2016, **4**.

12. Herbison REH: **Lessons in mind control: trends in research on the molecular mechanisms behind parasite-host behavioral manipulation**. *Front Ecol Evol* 2017, **5**:102.

13. Eigenbrode SD, Ding H, Shiel P, Berger PH: **Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae)**. *Proc Biol Sci* 2002, **269**:455-460.

14. Jiménez-Martínez ES, Bosque-Pérez NA, Berger PH, Zemetra RS, Ding H, Eigenbrode SD: **Volatile cues influence the response of *Rhopalosiphum padi* (Homoptera: Aphididae) to Barley yellow dwarf virus–infected transgenic and untransformed wheat**. *Environ Entomol* 2004, **33**:1207-1216.

15. Mauck KE, De Moraes CM, Mescher MC: **Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts**. *Proc Natl Acad Sci U S A* 2010, **107**:3600-3605.

16. Mauck KE, De Moraes CM, Mescher MC: **Evidence of local adaptation in plant virus effects on host–vector interactions**. *Integr Comp Biol* 2014, **54**:193-209.

17. Wu Y, Davis TS, Eigenbrode SD: **Aphid behavioral responses to virus-infected plants are similar despite divergent fitness effects**. *Entomol Exp Appl* 2014, **153**:246-255.

18. Chen G, Su Q, Shi X, Liu X, Peng Z, Zheng H, Xie W, Xu B, Wang S, Wu Q *et al.*: **Odor, not performance, dictates *Bemisia tabaci*'s selection between healthy and virus infected plants**. *Front Physiol* 2017, **8**:146.

19. Carmo-Sousa M, Moreno A, Plaza M, Garzo E, Fereres A: ***Cucurbit aphid-borne yellows virus* (CABYV) modifies the alighting, settling and probing behaviour of its vector *Aphis gossypii* favouring its own spread**. *Ann Appl Biol* 2016, **169**:284-297.

20. Fereres A, Peñaflor MFGV, Favaro CF, Azevedo KEX, Landi CH, Maluta NKP, Bento JMS, Lopes JRS: **Tomato infection by whitefly-transmitted circulative and non-circulative viruses induce contrasting changes in plant volatiles and vector behaviour**. *Viruses* 2016, **8**.

21. Peñaflor MFGV, Mauck KE, Alves KJ, De Moraes CM, Mescher MC: **Effects of single and mixed infections of *Bean pod mottle virus* and *Soybean mosaic virus* on host-plant chemistry and host–vector interactions**. *Funct Ecol* 2016, **30**:1648-1659.

22. McElhany P, Real LA, Power AG: **Vector preference and disease dynamics : a study of *Barley yellow dwarf virus***. *Ecology* 1995, **76**:444-457.

23. Sisterson MS: **Effects of insect-vector preference for healthy or infected plants on pathogen spread: insights from a model**. *J Econ Entomol* 2008, **101**:1-8.

24. • Shaw AK, Peace A, Power AG, Bosque-Pérez NA: **Vector population growth and condition-dependent movement drive the spread of plant pathogens**. *Ecology* 2017, **98**:2145-2157.

The authors developed a model based on vector-borne plant viruses and used it to evaluate the importance of various vector movement behavior and life history parameters for pathogen spread. Their results suggest that vector population growth rate has the largest impact on the rate of pathogen spread, followed by rate of vector dispersal from infected hosts/hosts of the same infection status. These results lend credence to the idea that virus-induced changes in vector performance and behavior have significant epidemiological implications and warrant further study.

25. Maris PC, Joosten NN, Goldbach RW, Peters D: ***Tomato spotted wilt virus* infection improves host suitability for its vector *Frankliniella occidentalis***. *Phytopathology* 2004, **94**:706-711.

26. Shrestha D, McAuslane HJ, Adkins ST, Smith HA, Dufault N, Colee J, Webb SE: **Host-mediated effects of semipersistently transmitted *Squash vein yellowing virus* on Sweetpotato whitefly (Hemiptera: Aleyrodidae) behavior and fitness**. *J Econ Entomol* 2017, **110**:1433-1441 http://dx.doi.org/10.1093/jee/tox161.

27. Maluta NKP, Fereres A, Lopes JRS: **Settling preferences of the whitefly vector *Bemisia tabaci* on infected plants varies with virus family and transmission mode**. *Entomol Exp Appl* 2017, **165**:138-147.

28. Chesnais Q, Couty A, Uzest M, Brault V, Ameline A: **Plant infection by two different viruses induce contrasting changes of vectors fitness and behavior**. *Insect Sci* 2019, **26**:86-96 http://dx.doi.org/10.1111/1744-7917.12508.

29. Daimei G, Raina HS, Devi PP, Saurav GK, Renukadevi P, Malathi VG, Senthilraja C, Mandal B, Rajagopal R: **Influence of *Groundnut bud necrosis virus* on the life history traits and feeding preference of its vector, *Thrips palmi***. *Phytopathology* 2017, **107**:1440-1445.

30. Jiu M, Zhou X-P, Tong L, Xu J, Yang X, Wan F-H, Liu S-S: **Vector-virus mutualism accelerates population increase of an invasive whitefly**. *PLoS One* 2007, **2**:e182.

31. Belliure B, Janssen A, Maris PC, Peters D, Sabelis MW: **Herbivore arthropods benefit from vectoring plant viruses**. *Ecol Lett* 2005, **8**:70-79.

32. Sun Y-C, Pan L-L, Ying F-Z, Li P, Wang X-W, Liu S-S: **Jasmonic acid-related resistance in tomato mediates interactions**

between whitefly and whitefly-transmitted virus. *Sci Rep* 2017, **7**:566.

33. Wang J, Bing X-L, Li M, Ye G-Y, Liu S-S: **Infection of tobacco plants by a begomovirus improves nutritional assimilation by a whitefly**. *Entomol Exp Appl* 2012, **144**:191-201.

34. Davis TS, Wu Y, Eigenbrode SD: **The effects of *Bean leafroll virus* on life history traits and host selection behavior of specialized pea aphid (*Acyrthosiphon pisum*, Hemiptera: Aphididae) genotypes**. *Environ Entomol* 2017, **46**:nvw150.

35. Castle SJ, Berger PH: **Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship**. *Entomol Exp Appl* 1993, **69**:51-60.

36. Mauck KE: **Variation in virus effects on host plant phenotypes and insect vector behavior: what can it teach us about virus evolution?** *Curr Opin Virol* 2016, **21**:114-123.

37. Donaldson JR, Gratton C: **Antagonistic effects of soybean viruses on soybean aphid performance**. *Environ Entomol* 2007, **36**:918-925.

38. Mauck KE, De Moraes CM, Mescher MC: **Biochemical and physiological mechanisms underlying effects of *Cucumber mosaic virus* on host-plant traits that mediate transmission by aphid vectors**. *Plant Cell Environ* 2014, **37**:1427-1439.

39. Blua MJ, Perring TM: **Effects of *Zucchini yellow mosaic virus* on colonization and feeding behavior of *Aphis gossypii* (Homoptera: Aphididae) alatae**. *Environ Entomol* 1992, **21**:578-585.

40. Wosula EN, Davis JA, Clark CA: **Stylet penetration behaviors of *Myzus persicae* (Hemiptera: Aphididae) on four *Ipomoea* spp. infected or noninfected with sweet potato potyviruses**. *J Econ Entomol* 2014, **107**:538-545.

41. Carmo-Sousa M, Moreno A, Garzo E, Fereres A: **A non-persistently transmitted-virus induces a pull–push strategy in its aphid vector to optimize transmission and spread**. *Virus Res* 2014, **186**:38-46.

42. Westwood JH, Groen SC, Du Z, Murphy AM, Anggoro DT, Tungadi T, Luang-In V, Lewsey MG, Rossiter JT, Powell G *et al.*: **A trio of viral proteins tunes aphid-plant interactions in *Arabidopsis thaliana***. *PLoS One* 2013, **8**:e83066.

43. Ellsbury MM, Pratt RG, Knight WE: **Effects of single and combined infection of arrowleaf clover with *Bean yellow mosaic virus* and a *Phytophthora* sp. on reproduction and colonization by pea aphids (Homoptera: Aphididae)**. *Environ Entomol* 1985, **14**:356-359.

44. Martin B, Collar JL, Tjallingii WF, Fereres A: **Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses**. *J Gen Virol* 1997, **78**:2701-2705.

45. Wang RY, Ghabrial SA: **Effect of aphid behavior on efficiency of transmission of *Soybean mosaic virus* by the soybean-colonizing aphid, *Aphis glycines***. *Plant Dis* 2002, **86**:1260-1264.

46. Mondal S, Wenninger EJ, Hutchinson PJS, Weibe MA, Eigenbrode SD, Bosque-Pérez NA: **Contribution of noncolonizing aphids to *Potato virus Y* prevalence in potato in Idaho**. *Environ Entomol* 2016, **45**:1445-1462.

47. Lozano-Durán R, Rosas-Díaz T, Gusmaroli G, Luna AP, Taconnat L, Deng XW, Bejarano ER: **Geminiviruses subvert ubiquitination by altering CSN-mediated derubylation of SCF E3 ligase complexes and inhibit jasmonate signaling in *Arabidopsis thaliana***. *Plant Cell* 2011, **23**:1014-1032.

48. Qiu Y, Zhang Y, Wang C, Lei R, Wu Y, Li X, Zhu S: ***Cucumber mosaic virus* coat protein induces the development of chlorotic symptoms through interacting with the chloroplast ferredoxin I protein**. *Sci Rep* 2018, **8**:1205.

49. Zhang X, Yuan Y-R, Pei Y, Lin S-S, Tuschl T, Patel DJ, Chua N-H: **Cucumber mosaic virus-encoded 2b suppressor inhibits *Arabidopsis* Argonaute1 cleavage activity to counter plant defense**. *Genes Dev* 2006, **20**:3255-3268.

50. González I, Martínez L, Rakitina DV, Lewsey MG, Atencio FA, Llave C, Kalinina NO, Carr JP, Palukaitis P, Canto T: ***Cucumber mosaic virus* 2b protein subcellular targets and interactions: their significance to RNA silencing suppressor activity**. *Mol Plant Microbe Interact* 2010, **23**:294-303.

51. Hofius D, Herbers K, Melzer M, Omid A, Tacke E, Wolf S, Sonnewald U: **Evidence for expression level-dependent modulation of carbohydrate status and viral resistance by the *Potato leafroll virus* movement protein in transgenic tobacco plants**. *Plant J* 2001, **28**:529-543.

52. Rinne PLH, van den Boogaard R, Mensink MGJ, Kopperud C, Kormelink R, Goldbach R, van der Schoot C: **Tobacco plants respond to the constitutive expression of the tospovirus movement protein NS(M) with a heat-reversible sealing of plasmodesmata that impairs development**. *Plant J* 2005, **43**:688-707.

53. Kong LJ, Orozco BM, Roe JL, Nagar S, Ou S, Feiler HS, Durfee T, Miller AB, Gruissem W, Robertson D *et al.*: **A geminivirus replication protein interacts with the retinoblastoma protein through a novel domain to determine symptoms and tissue specificity of infection in plants**. *EMBO J* 2000, **19**:3485-3495.

54. Zhang Z, Chen H, Huang X, Xia R, Zhao Q, Lai J, Teng K, Li Y, Liang L, Du Q *et al.*: **BSCTV C2 attenuates the degradation of SAMDC1 to suppress DNA methylation-mediated gene silencing in *Arabidopsis***. *Plant Cell* 2011, **23**:273-288.

55. Pazhouhandeh M, Dieterle M, Marrocco K, Lechner E, Berry B, Brault V, Hemmer O, Kretsch T, Richards KE, Genschik P *et al.*: **F-box-like domain in the polerovirus protein P0 is required for silencing suppressor function**. *Proc Natl Acad Sci U S A* 2006, **103**:1994-1999.

56. Geri C, Love AJ, Cecchini E, Barrett SJ, Laird J, Covey SN, Milner JJ: ***Arabidopsis* mutants that suppress the phenotype induced by transgene-mediated expression of *Cauliflower mosaic virus* (CaMV) gene VI are less susceptible to CaMV-infection and show reduced ethylene sensitivity**. *Plant Mol Biol* 2004, **56**:111-124.

57. Love AJ, Geri C, Laird J, Carr C, Yun B-W, Loake GJ, Tada Y, Sadanandom A, Milner JJ: ***Cauliflower mosaic virus* protein P6 inhibits signaling responses to salicylic acid and regulates innate immunity**. *PLoS One* 2012, **7**:e47535.

58. Love AJ, Yun BW, Laval V, Loake GJ, Milner JJ: ***Cauliflower mosaic virus*, a compatible pathogen of *Arabidopsis*, engages three distinct defense-signaling pathways and activates rapid systemic generation of reactive oxygen species**. *Plant Physiol* 2005, **139**:935-948.

59. • Zhao W, Yang P, Kang L, Cui F: **Different pathogenicities of *Rice stripe virus* from the insect vector and from viruliferous plants**. *New Phytol* 2016, **210**:196-207.
Infection of plants by a circulative propagative virus that had not passed through an insect vector resulted in different levels of expression of many viral genes, produced different visual symptoms, and, in contrast to insect-derived virus, did not damage the host plant's chloroplasts nor interfere with transcription of photosynthesis-related genes. The latter may be due to differences in the ability of the viral Sp gene to interact with the necessary host plant components.

60. Simón-Buela L, Guo HS, García JA: **Long sequences in the 5′ noncoding region of *Plum pox virus* are not necessary for viral infectivity but contribute to viral competitiveness and pathogenesis**. *Virology* 1997, **233**:157-162.

61. Lough TJ, Lee RH, Emerson SJ, Forster RLS, Lucas WJ: **Functional analysis of the 5′ untranslated region of potexvirus RNA reveals a role in viral replication and cell-to-cell movement**. *Virology* 2006, **351**:455-465.

62. Pallas V, García JA: **How do plant viruses induce disease? Interactions and interference with host components**. *J Gen Virol* 2011, **92**:2691-2705.

63. Havelda Z, Hornyik C, Válóczi A, Burgyán J: **Defective interfering RNA hinders the activity of a tombusvirus-encoded posttranscriptional gene silencing suppressor**. *J Virol* 2005, **79**:450-457.

64. Shimura H, Pantaleo V, Ishihara T, Myojo N, Inaba J-I, Masuta C: **A viral satellite RNA induces yellow symptoms on tobacco by targeting a gene involved in chlorophyll biosynthesis using the RNA silencing machinery**. *PLoS Pathog* 2011, **7**:e1002021.

65. Gallet R, Michalakis Y, Blanc S: **Vector-transmission of plant viruses and constraints imposed by virus-vector interactions**. *Curr Opin Virol* 2018, **33**:144-150.

66. Whitfield AE, Kumar NKK, Rotenberg D, Ullman DE, Wyman EA, Zietlow C, Willis DK, German TL: **A soluble form of the *Tomato spotted wilt virus* (TSWV) glycoprotein G(N) (G(N)-S) inhibits transmission of TSWV by *Frankliniella occidentalis***. *Phytopathology* 2008, **98**:45-50.

67. Sin S-H, McNulty BC, Kennedy GG, Moyer JW: **Viral genetic determinants for thrips transmission of *Tomato spotted wilt virus***. *Proc Natl Acad Sci U S A* 2005, **102**:5168-5173.

68. Whitfield AE, Ullman DE, German TL: **Expression and characterization of a soluble form of *Tomato spotted wilt virus* glycoprotein GN**. *J Virol* 2004, **78**:13197-13206.

69. Ammar E-D, Tsai C-W, Whitfield AE, Redinbaugh MG, Hogenhout SA: **Cellular and molecular aspects of rhabdovirus interactions with insect and plant hosts**. *Annu Rev Entomol* 2009, **54**:447-468.

70. Lu G, Li S, Zhou C, Qian X, Xiang Q, Yang T, Wu J, Zhou X, Zhou Y, Ding XS *et al.*: **Tenuivirus uses a molecular bridge strategy to overcome insect midgut barriers for virus persistent transmission**. *bioRxiv* 2018 http://dx.doi.org/10.1101/404657.

71. • Ogada PA, Kiirika LM, Lorenz C, Senkler J, Braun H-P, H-M Poehling: **Differential proteomics analysis of *Frankliniella occidentalis* immune response after infection with *Tomato spotted wilt virus* (Tospovirus)**. *Dev Comp Immunol* 2017, **67**:1-7.

Researchers used 2D-IEF/SDS-PAGE followed by mass spectrometry to identify proteins differentially expressed in TSWV-infected thrips. This revealed changes in the expression of many proteins, the majority of which are related to the innate immune response.

72. Zhou F, Pu Y, Wei T, Liu H, Deng W, Wei C, Ding B, Omura T, Li Y: **The P2 capsid protein of the nonenveloped rice dwarf phytoreovirus induces membrane fusion in insect host cells**. *Proc Natl Acad Sci U S A* 2007, **104**:19547-19552.

73. Mao Q, Zheng S, Han Q, Chen H, Ma Y, Jia D, Chen Q, Wei T: **New model for the genesis and maturation of viroplasms induced by fijiviruses in insect vector cells**. *J Virol* 2013, **87**:6819-6828.

74. Wu W, Zheng L, Chen H, Jia D, Li F, Wei T: **Nonstructural protein NS4 of *Rice stripe virus* plays a critical role in viral spread in the body of vector insects**. *PLoS One* 2014, **9**:e88636.

75. • Geng L, Qian L-X, Shao R-X, Liu Y-Q, Liu S-S, Wang X-W: **Transcriptome profiling of whitefly guts in response to *Tomato yellow leaf curl virus* infection**. *Virol J* 2018, **15**:14.

Using illumina sequencing, the authors demonstrated that 5207 genes were differentially expressed in the guts of whiteflies infected with TYLCV compared to those of non-viruliferous whiteflies. Notably, this included genes related to transmembrane transport, cell cycle regulation, DNA repair, intracellular signaling, and defense response.

76. Brault V, Périgon S, Reinbold C, Erdinger M, Scheidecker D, Herrbach E, Richards K, Ziegler-Graff V: **The polerovirus minor capsid protein determines vector specificity and intestinal tropism in the aphid**. *J Virol* 2005, **79**:9685-9693.

77. Brault V, Tanguy S, Reinbold C, Le Trionnaire G, Arneodo J, Jaubert-Possamai S, Guernec G, Tagu D: **Transcriptomic analysis of intestinal genes following acquisition of *Pea enation mosaic virus* by the pea aphid *Acyrthosiphon pisum***. *J Gen Virol* 2010, **91**:802-808.

78. • Mulot M, Monsion B, Boissinot S, Rastegar M, Meyer S, Bochet N, Brault V: **Transmission of *Turnip yellows virus* by *Myzus persicae* is reduced by feeding aphids on double-stranded RNA targeting the ephrin receptor protein**. *Front Microbiol* 2018, **9**:457.

Using protein–protein interaction assays in a yeast system, the researchers demonstrated that a membrane-bound Ephrin receptor produced in the aphid vector *Myzus persicae* is involved in transmission of the circulative *Polerovirus*, *Turnip yellows virus* (TuYV). Delivery of dsRNA targeting the Ephrin receptor mRNA significantly reduced virus acquisition. The functions of Ephrin receptors in aphids are not known, but in humans they play critical roles in the maintenance of many cellular processes. In addition to a role in TuYV invasion of vector tissues, the aphid Ephrin receptor may be involved in initiation of cell signaling and expression cascades in response to the presence of virions in the aphid digestive tract.

79. Lewsey M, Surette M, Robertson FC, Ziebell H, Choi SH, Ryu KH, Canto T, Palukaitis P, Payne T, Walsh JA *et al.*: **The role of the *Cucumber mosaic virus* 2b protein in viral movement and symptom induction**. *Mol Plant Microbe Interact* 2009, **22**:642-654.

80. Lewsey MG, González I, Kalinina NO, Palukaitis P, Canto T, Carr JP: **Symptom induction and RNA silencing suppression by the *Cucumber mosaic virus* 2b protein**. *Plant Signal Behav* 2010, **5**:705-708.

81. Casteel CL, Yang C, Nanduri AC, De Jong HN, Whitham SA, Jander G: **The NIa-Pro protein of *Turnip mosaic virus* improves growth and reproduction of the aphid vector, *Myzus persicae* (green peach aphid)**. *Plant J* 2014, **77**:653-663.

82. Casteel CL, De Alwis M, Bak A, Dong H, Whitham SA, Jander G: **Disruption of ethylene responses by *Turnip mosaic virus* mediates suppression of plant defense against the green peach aphid vector**. *Plant Physiol* 2015, **169**:209-218.

83. •• Bak A, Cheung AL, Yang C, Whitham SA, Casteel CL: **A viral protease relocalizes in the presence of the vector to promote vector performance**. *Nat Commun* 2017, **8**:14493.

By examining whole-virus infection as well as the effects of transgenically expressed viral proteins, this study demonstrated that aphid feeding induces the NIa-Pro protein of TuMV to relocalize from the cytoplasm or nucleus to the vacuole. This induces a suite of effects on the host plant linked to perturbations in ethylene signaling that enhance aphid performance. As all potyviruses contain an NIa-Pro, this may be a conserved mechanism underlying host-mediated vector manipulation by these viruses.

84. •• Wu D, Qi T, Li W-X, Tian H, Gao H, Wang J, Ge J, Yao R, Ren C, Wang X-B *et al.*: **Viral effector protein manipulates host hormone signaling to attract insect vectors**. *Cell Res* 2017, **27**:402-415.

This study used transgenic plants expressing viral proteins to identify the*Cucumber mosaic virus* (CMV) 2b protein as the component responsible for enhancing the attractiveness of CMV-infected plants to vectors. *In vitro* pull-down assays showed that 2b binds directly to components of the JA signaling pathway, inhibiting expression of JA-regulated genes involved in herbivore defense. Importantly, the 2b protein role in inducing aphid attraction is independent of its role in suppressing host antiviral RNA silencing.

85. Westwood JH, Lewsey MG, Murphy AM, Tungadi T, Bates A, Gilligan CA, Carr JP: **Interference with jasmonic acid-regulated gene expression is a general property of viral suppressors of RNA silencing but only partly explains virus-induced changes in plant-aphid interactions**. *J Gen Virol* 2014, **95**:733-739.

86. Ziebell H, Murphy AM, Groen SC, Tungadi T, Westwood JH, Lewsey MG, Moulin M, Kleczkowski A, Smith AG, Stevens M *et al.*: ***Cucumber mosaic virus* and its 2b RNA silencing suppressor modify plant-aphid interactions in tobacco**. *Sci Rep* 2011, **1**:187.

87. Tungadi T, Groen SC, Murphy AM, Pate AE, Iqbal J, Bruce TJA, Cunniffe NJ, Carr JP: ***Cucumber mosaic virus* and its 2b protein alter emission of host volatile organic compounds but not aphid vector settling in tobacco**. *Virol J* 2017, **14**:91.

88. Li R, Weldegergis BT, Li J, Jung C, Qu J, Sun Y, Qian H, Tee C, van Loon JJA, Dicke M *et al.*: **Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance**. *Plant Cell* 2014, **26**:4991-5008.

89. Zhang T, Luan J-B, Qi J-F, Huang C-J, Li M, Zhou X-P, Liu S-S: **Begomovirus–whitefly mutualism is achieved through repression of plant defences by a virus pathogenicity factor**. *Mol Ecol* 2012, **21**:1294-1304.

90. Liu B, Preisser EL, Shi X, Wu H, Li C, Xie W, Wang S, Wu Q, Zhang Y: **Plant defense negates pathogen manipulation of vector behavior**. *Funct Ecol* 2017, **31**:1574-1581 http://dx.doi.org/10.1111/1365-2435.12872.

91. Yang J-Y, Iwasaki M, Machida C, Machida Y, Zhou X, Chua N-H: **βC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses**. *Genes Dev* 2008, **22**:2577.

92. Wasternack C, Hause B: **Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany**. *Ann Bot* 2013, **111**:1021-1058.

93. Lafferty KD, Shaw JC: **Comparing mechanisms of host manipulation across host and parasite taxa**. *J Exp Biol* 2013, **216**:56-66.

94. Gray S, Cilia M, Ghanim M: **Circulative, "nonpropagative" virus transmission: an orchestra of virus-, insect-, and plant-derived instruments**. *Adv Virus Res* 2014, **89**:141-199.

95. Montero-Astúa M, Ullman DE, Whitfield AE: **Salivary gland morphology, tissue tropism and the progression of tospovirus infection in *Frankliniella occidentalis***. *Virology* 2016, **493**:39-51.

96. Dáder B, Then C, Berthelot E, Ducousso M, Ng JCK, Drucker M: **Insect transmission of plant viruses: multilayered interactions optimize viral propagation**. *Insect Sci* 2017, **24**:929-946 http://dx.doi.org/10.1111/1744-7917.12470.

97. • Schneweis DJ, Whitfield AE, Rotenberg D: **Thrips developmental stage-specific transcriptome response to tomato spotted wilt virus during the virus infection cycle in *Frankliniella occidentalis*, the primary vector**. *Virology* 2017, **500**:226-237.

The authors used a brief larval TSWV acquisition period followed by feeding on healthy, non-host plant tissue to obtain thrips transcriptomes at four different time points post-infection. This temporal tracking revealed that the effect of TSWV on thrips transcript accumulation varies greatly between developmental stages, consistent with previous work demonstrating differences in tissue tropism and TSWV acquisition efficiency depending on life stage.

98. • Liu B, Preisser EL, Chu D, Pan H, Xie W, Wang S, Wu Q, Zhou X, Zhang Y: **Multiple forms of vector manipulation by a plant-Infecting virus: *Bemisia tabaci* and *Tomato yellow leaf curl virus***. *J Virol* 2013, **87**:4929-4937.

Here, transgenic tomato plants varying in expression levels of jasmonate as well as exogenous jasmonate applications were used in conjunction with EPG measuring aphid behavior to determine that high levels of JA decrease TYLCV infection of new plants by interfering with manipulation of vector behavior. This is the first evidence that variation in plant traits, such as JA-mediated plant responses, can alter vector-parasite-host interactions.

99. Moreno-Delafuente A, Garzo E, Moreno A, Fereres A: **A plant virus manipulates the behavior of its whitefly vector to enhance its transmission efficiency and spread**. *PLoS One* 2013, **8**:e61543.

100. Maluta NKP, Garzo E, Moreno A, Lopes JRS, Fereres A: ***Tomato yellow leaf curl virus* benefits population growth of the Q biotype of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)**. *Neotrop Entomol* 2014, **43**:385-392.

101. Legarrea S, Barman A, Marchant W, Diffie S, Srinivasan R: **Temporal effects of a Begomovirus infection and host plant resistance on the preference and development of an insect vector, *Bemisia tabaci*, and implications for epidemics**. *PLoS One* 2015, **10**:e0142114.

102. Stafford CA, Walker GP, Ullman DE: **Infection with a plant virus modifies vector feeding behavior**. *Proc Natl Acad Sci U S A* 2011, **108**:9350-9355.

103. Keough S, Han J, Shuman T, Wise K, Nachappa P: **Effects of *Soybean vein necrosis virus* on life history and host preference of its vector, *Neohydatothrips variabilis*, and evaluation of vector status of *Frankliniella tritici* and *Frankliniella fusca***. *J Econ Entomol* 2016, **109**:1979-1987.

104. Xu H, He X, Zheng X, Yang Y, Tian J, Lu Z: ***Southern rice black-streaked dwarf virus* (SRBSDV) directly affects the feeding and reproduction behavior of its vector, *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae)**. *Virol J* 2014, **11**:55.

105. Lu G, Zhang T, He Y, Zhou G: **Virus altered rice attractiveness to planthoppers is mediated by volatiles and related to virus titre and expression of defence and volatile-biosynthesis genes**. *Sci Rep* 2016, **6**:38581.

106. Wang Q, Li J, Dang C, Chang X, Fang Q, Stanley D, Ye G: ***Rice dwarf virus* infection alters green rice leafhopper host preference and feeding behavior**. *PLoS One* 2018, **13**:e0203364.

107. Wan G, Jiang S, Wang W, Li G, Tao X, Pan W, Sword GA, Chen F: ***Rice stripe virus* counters reduced fecundity in its insect vector by modifying insect physiology, primary endosymbionts and feeding behavior**. *Sci Rep* 2015, **5**:12527.

108. Bencharki B, Boissinot S, Revollon S, Ziegler-Graff V, Erdinger M, Wiss L, Dinant S, Renard D, Beuve M, Lemaitre-Guillier C *et al.*: **Phloem protein partners of *Cucurbit aphid borne yellows virus*: possible involvement of phloem proteins in virus transmission by aphids**. *Mol Plant Microbe Interact* 2010, **23**:799-810.

109. Shrestha A, Champagne DE, Culbreath AK, Rotenberg D, Whitfield AE, Srinivasan R: **Transcriptome changes associated with *Tomato spotted wilt virus* infection in various life stages of its thrips vector, *Frankliniella fusca* (Hinds)**. *J Gen Virol* 2017, **98**:2156-2170.

110. Kaur N, Chen W, Zheng Y, Hasegawa DK, Ling K-S, Fei Z, Wintermantel WM: **Transcriptome analysis of the whitefly, *Bemisia tabaci* MEAM1 during feeding on tomato infected with the crinivirus, *Tomato chlorosis virus*, identifies a temporal shift in gene expression and differential regulation of novel orphan genes**. *BMC Genomics* 2017, **18**:370.

111. Hasegawa DK, Chen W, Zheng Y, Kaur N, Wintermantel WM, Simmons AM, Fei Z, Ling K-S: **Comparative transcriptome analysis reveals networks of genes activated in the whitefly, *Bemisia tabaci* when fed on tomato plants infected with *Tomato yellow leaf curl virus***. *Virology* 2018, **513**:52-64.

112. Blanc S, Drucker M, Uzest M: **Localizing viruses in their insect vectors**. *Annu Rev Phytopathol* 2014, **52**:403-425.

113. Alexander MM, Mohr JP, DeBlasio SL, Chavez JD, Ziegler-Graff V, Brault V, Bruce JE, Heck MC: **Insights in luteovirid structural biology guided by chemical cross-linking and high resolution mass spectrometry**. *Virus Res* 2017, **241**:42-52 http://dx.doi.org/10.1016/j.virusres.2017.05.005.

114. Stafford-Banks CA, Yang LH, McMunn MS, Ullman DE: **Virus infection alters the predatory behavior of an omnivorous vector**. *Oikos* 2014, **123**:1384-1390.